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Short communication

Morphometric characterization of *Dinophysis acuminata*/*D. sacculus* complex in Guanabara Bay, Brazil

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Abstract: Most studies of *Dinophysis acuminata* in Brazil are for the southern region, where blooms are recurrent. In 2016, the presence of *D. acuminata* caused the first-ever production and consumption of species of mollusks commercial embargo from the state of Sao Paulo, Southeast Brazil. Potentially toxic species of *Dinophysis* have been reported in Guanabara Bay (GB) but only occasionally and in low densities. The present note is the first report of a high-density event (~10^5 cells L^{-1}) of *D. acuminata*/*D. sacculus* complex in GB. *D. acuminata*/*D. sacculus* complex species were identified using scanning-electron and inverted-light microscopy. Most of the studied cells possessed a dorsally convex hyposomal plate and had dimensions typical of *D. acuminata*. However, the observed association with warmer and less saline estuarine waters would indicate that the species could be *D. sacculus*. Whatever the case, based on the high cell densities observed here, we recommend a continued monitoring for *Dinophysis* presence in GB.
Keywords: *dinoflagellates, Dinophysis acuminata, Dinophysis sacculus, eutrophic marine ecosystem, South Atlantic Central Water*

Caracterização morfométrica de dinoflagelados do Complexo *Dinophysis acuminata*/*D. saculus* na Baía de Guanabara, Brasil

Resumo: A maioria dos estudos sobre *Dinophysis* acuminata no Brasil ocorreram na região sul, onde as florações são recorrentes. Em 2016, a presença de *D. acuminata* causou o primeiro embargo comercial da produção e consumo de espécies de moluscos do estado de São Paulo, sudeste do Brasil. Várias espécies de microalgas potencialmente nocivas foram relatadas na Baía de Guanabara (BG), incluindo espécies tóxicas de *Dinophysis*, mas estas foram reportadas apenas como ocasionais e em baixas densidades. A presente nota é o primeiro relato de um evento de alta densidade (~ 10^5 células L^{-1}) do complexo *D. acuminata*/*D. saculus* na BG. As espécies foram identificadas através de microscopia eletrônica de varredura e de campo claro. A maioria das células estudadas possuía uma placa hipossômica dorsalmente convexa, e tinha dimensões típicas de *D. acuminata*. No entanto, a associação observada com águas estuarinas mais quentes e menos salinas indicaria que a espécie seria *D. saculus*. Qualquer que seja o caso, com base nas altas densidades observadas aqui, recomendamos o monitoramento contínuo da presença de *Dinophysis* na BG.
Palavras-chave: *dinoflagelados, Dinophysis acuminata, Dinophysis saculus, ecossistema marinho eutrófico, Água Central do Atlântico Sul.*

Introduction

The genus *Dinophysis* Ehrenberg includes species with a diverse morphology and different trophic strategies (autotrophic, heterotrophic and mixotrophic) (Zingone et al. 1998). Some of these species are potential producers of phytotoxin and can be responsible for human intoxication events, even in densities as low as $<10^2$ cells·L^{-1}, which are rarely detected by quantitative methods (Reguera et al. 2012). *Dinophysis acuminata* Claparède & Lachmann (Hattenrath-Lehmann et al. 2015) has received significant attention because inputs of nutrients and organic matter can promote both its toxicity and growth. Nevertheless, this species belongs to the "*D. acuminata* complex", which contains taxa that are difficult to discriminate with conventional microscopy due to morphological variability (Reguera et al. 2012). One such case is the pair *D. acuminata* Claparède & Lachmann/*D. sacculus* F. Stein (Zingone et al. 1998), which can co-occur (Reguera et al. 2012). Both species have been associated with diarrhetic shellfish poisoning (DSP) events (Reguera et al. 2012, and references, García-Altares et al. 2016).

In Brazil, most of the studies on *D. acuminata* have occurred in the South region, due to its great importance for oyster and mussel cultivation. (Mafra-Junior et al. 2006, Mello et al. 2010, Simões et al. 2014, Tibiriçá et al. 2015). Natural blooms of this species along the southern Brazilian coast lead to recurrent commercial embargos of cultivated species, resulting in important economic losses (Simões et al. 2014). From May to July 2016, *D. acuminata* was reported along the coast from Santa Catarina to São Paulo in densities that led to the first-ever commercial embargo of the production and consumption of oysters and mussels by the health authorities of the state of São Paulo (A Tribuna 2016).

Although *D. acuminata* has been detected along Rio de Janeiro's coast, blooms have not been reported yet. At Sepetiba Bay on the southern coast of Rio de Janeiro, *D. acuminata* was found to be dominant among the five species of the genus detected, but both cell densities and toxin concentrations on mussels were lower than the limit allowed by law (Ferreira et al. 2010, Brasil 2012). Guanabara Bay (GB) encompasses many more municipalities than the city of Rio de Janeiro, being the second largest bay of the Brazilian coast, and is historically under intense eutrophication, thus a program of continuous monitoring of planktonic species should be implemented (Fistarol et al. 2015). Several potentially harmful microalgal species have been reported at high densities in GB: *Scrippsiella trochoidea* (Stein) Loeblich (Villac & Tenenbaum 2010), *Pseudo-nitzschia* H. Peragallo spp. (Rezende et al. 2015), filamentous cyanobacteria, *Prorocentrum* Ehrenberg spp. (Villac & Tenenbaum, 2010, Rezende et al. 2015), and *Chattonella* B.Biecheler spp. (Fistarol et al. 2015). The potentially toxic species of the genus *Dinophysis* (i.e. *D. acuminata/ D. sacculus)*, however, have been described as only occasional and in low densities (Rezende et al. 2015). Nonetheless, several studies have recommended the implementation of protocols able to detect *Dinophysis* sp. at low-densities ($<10^2$ cells L^{-1}) in the water column serving as an early warning system (Reguera et al. 2014).

The present work is the first report of a bloom of *Dinophysis* in Guanabara Bay, Rio de Janeiro (Brazil), and a morphological and morphometric study of the analyzed cells is provided.

Material and Methods

Surveys were performed on September 1st, 2015, along a transect of eight sampling points from Paquetá Island (inner region) to Rasa Island (outer region) along the dredged channel of Guanabara Bay (22°80'S; 43°14'W) (Figure 1). Phytoplankton samples were collected, both during ebb and during flood tides, with Niskin bottles at sub-surface and near the bottom. Sub-samples of 250 mL were fixed with Lugol´s solution and preserved in the dark, at room temperature, until laboratory analysis. At laboratory, aliquots of 5 – 10 mL were prepared according to the Utermölh (1958) method and examined and photographed under 200 x and 400 x magnification using a ZEISS® Axiovert A1 inverted microscope and an ZEISS® AxioCAM 105 camera. Bright field (BF) and phase contrast (PH) were used for identification. Images of the different organisms identified were captured with the AxioCAM 105 camera and processed with the software Zen (Blue Edition; Carl ZEISS®). The images of 55 organisms were used to measure the morphological parameters (length, width and the hidden dimension) of the cells of *D. acuminata/D. sacculus* complex. Cell surface and cell volume were calculated according with the equations described in Sun & Liu (2003). Samples were prepared for scanning electron microscopy (SEM) by gentle filtration of 20 ml through 0.2 µm pore-size Nuclepore membranes. Salt was removed by washing the filters with small amounts of deionized water. The filters were dried, mounted on stubs with double-sided sticky tape and vacuum coated with graphite. The samples were examined with a JEOL JSM 6510LV scanning electron microscope.

The relationships among phytoplanktonic cell densities and abiotic variables were determined using Pearson's correlation (Software Statistica® by Statsoft) (Cassie, 1962).

Results

Water temperature was higher in the inner region (19.9 – 25.1 °C) than in the outer region (16.1 – 19.8 °C) of the bay. Salinity ranged from 24.2 to 35.2 in the inner region, and from 35.3 to 36.0 in the outer. Total chlorophyll ranged from 2.04 to 45.03 mg m^{-3} in the inner region, and from 0.45 to 7.48 mg m^{-3} in the outer.

Phytoplankton densities were higher in the inner region ($2.8x10^5$ – $2.8x10^7$ cells L^{-1}) than in the outer region ($5.1x10^3$ – $4.1x10^6$ cells L^{-1}). Although they were not dominant, dinoflagellate densities ranged from $1.3x10^4$ – $2.2x10^6$ cells L^{-1} to $4.0x10^1$ – $3.9x10^5$ cells L^{-1}, in the inner and outer regions, respectively. Among the dinoflagellates, the genus *Prorocentum* was dominant (average > 50 %), both in the inner and outer regions. The contribution of the genus *Dinophysis* was lower than 10% on average (maximum 25%), with the *D. acuminata*/*D. sacculus* complex being dominant for this genus (maximum $1.2x10^5$ cells L^{-1} at inner portion) (Table 1). The cell density of the *D. acuminata*/*D. sacculus* complex was positively correlated with that of *P. dentatum* ($r=0.91$; $p<0.001$) and *P. micans* ($r=0.79$; $p<0.01$). The abundance of *D. acuminata/D. sacculus* complex was also positively correlated ($r= 0.72$; $p<0.001$) with temperature and negatively correlated ($r= -0.61$; $p<0.001$) with salinity.

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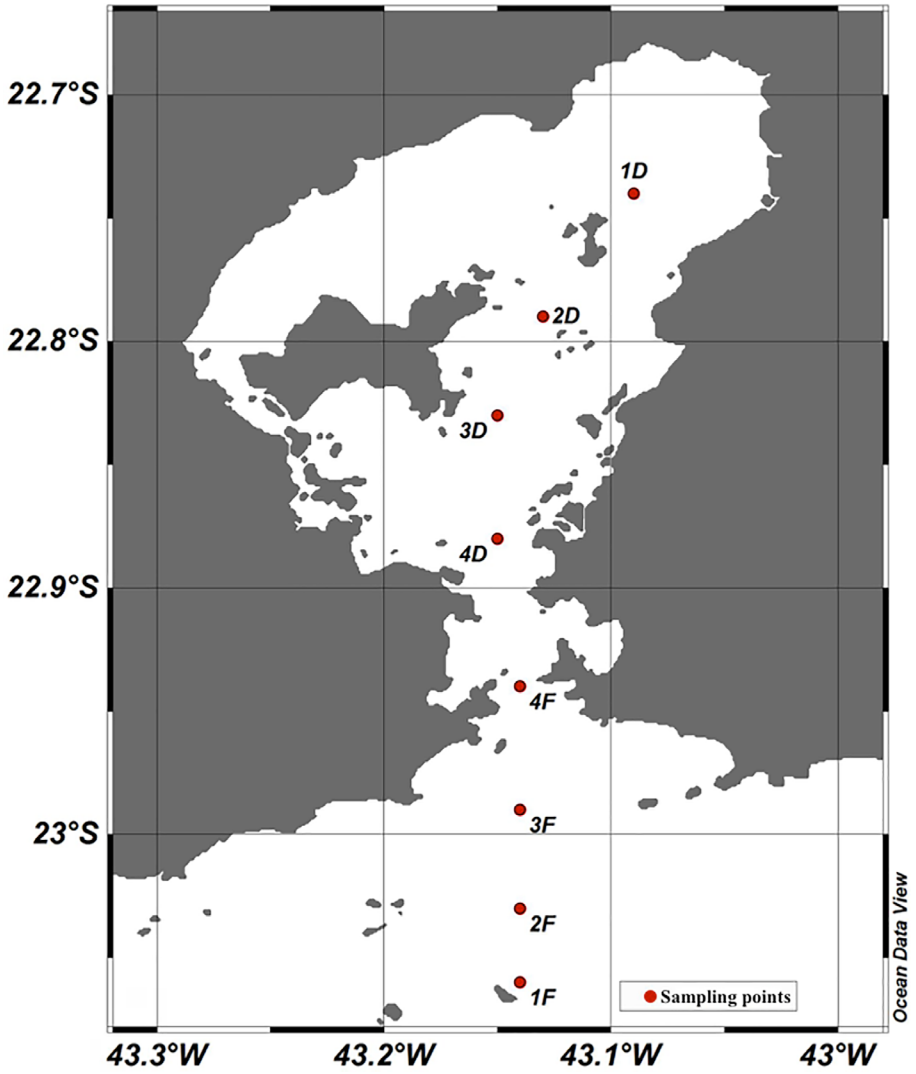

Figure 1. Study area - Guanabara Bay (Rio de Janeiro, Brazil). Sampling points (red dots) in a transect from the inner part of the bay (denoted by a number and letter D) to the inner continental shelf (denoted by a number and letter F).

Table 1. Average (av.), standard deviation (sd), minimum (min) and maximum (max) of cell densities (x10^3 cells L^{-1}) of *Dinophysis genera*, *Dinophysis acuminata*/*sacculus* complex, and Prorocentrum genera at inner and outer regions.

Region		***Dinophysis acuminata/ sacculus* complex**	***Prorocentrum micans***	***Prorocentrum dentatum***
inner	av.	22.57	210.84	339.73
	sd	29.34	332.52	330.74
	min	1.40	0.40	3.40
	max	115.55	1021.91	837.75
outer	av.	0.77	5.17	10.48
	sd	0.97	12.51	16.07
	min	0.00	0.00	0.00
	max	3.00	43.00	50.20

Cells of the *Dinophysis acumitata/D. sacculus* complex were 33.2 – 44.2 µm long, 20.3 – 32.3 µm wide, and 13.5 – 20.1 µm of hidden dimension (Table 2). The length/width (l/w) ratio varied from 1.2 to 1.9 and the Surface/Volume ratio ranged from 0.24 to 0.28. The shape of the cells was slightly convex, with a convex and sculptured sulcal platelet with three ribs, which was almost half of the hypothecal length (Figure 2a-g); this morphotype is intermediate between the two species of this complex.

Under SEM, small smooth pores were seen irregularly scattered on the surface of the hypotheca (Figure 2a). The number of pores distributed along 10 µm varies from six in the middle region of the hypotheca, to 12 near the cingular platelet (Figure 3a-b). Two rows of pores were observed on the sulcal platelet (Figure 3c).

Table 2. Average (av.), standard deviation (sd, minimum (min) and maximum (max) length, width, hidden dimension, length/width (l/w) ratio, surface (S), volume (V) and S/V ratio for the *Dinophysis acuminata*/*D. sacculus* complex at September 2015 (n=55).

***Dinophysis acuminata*/*D. sacculus* complex metrics**				
	av.	**sd**	**min**	**max**
length (µm)	38.8	1.9	33.2	44.2
width (µm)	25.1	2.7	20.3	32.3
hidden dimension (µm)	16.9	2.4	13.5	20.1
l/w ratio	1.56	0.14	1.17	1.90
S (μm^2)	2226	229	1709	2812
V (μm^3)	8648	1188	6085	11651
S/V	0.26	0.01	0.24	0.28

Discussion

During the winter of 2015, a high-abundance (~10^5-10^6 cells L^{-1}) event of dinoflagellates was observed at Guanabara Bay (Rio de Janeiro, Brazil). It was dominated by the genus *Prorocentrum* Ehrenberg, mainly the species *P. dentatum* F.Stein and *P. micans* Ehrenberg. Associated with these, the high cell densities of the *Dinophysis acuminata*/*D. sacculus* complex were also observed. The co-occurrence of *Dinophysis* and *Prorocentrum* blooms were previously reported by several studies (i.e. Reguera et al. 2012, Hattenrath-Lehmann et al. 2015), as well as the occurrence of a bloom of *Prorocentrum* after *Dinophysis* events (Campbell et al. 2010). *Prorocentrum micans*, which reached densities as high as 10^6 cells L^{-1} in the present work, was reported as highly abundant in warm and nutrient-rich waters, such as that of GB (Sahraoui et al. 2013). *Prorocentrum micans* was also reported as a producer of putative palytoxin and ovatoxin-a (Ignatiades & Gotsis-Skretas 2010), nevertheless, the frequent association of this species with DSP could be due to the presence of *D. acuminata* in densities too low to be detected (Reguera et al. 2014 and references therein).

Although *D. acuminata* is usually associated with colder waters and *D. sacculus* with warmer waters, the two species were reported coexisting in several coastal environments, probably due to the difficulty in distinguishing their cells (Zingone et al. 1998). Thus, in GB, the *Dinophysis* complex was clearly associated with the warmer and less saline waters of the inner estuary. In a study in southern Brazil, Haraguchi and Odebrecht (2010) reported high abundances of *D. acuminata* (~10^4 cells L^{-1}) associated with intrusions of a cold and salty water mass, the South Atlantic Central Water (SACW), while other species of *Dinophysis*, such as *D. fortii* Pavillard, were associated with warmer waters. In the present work, the influence of the SACW was especially observed in the outer region of GB (*data not published*), where lower densities of the *D. acuminata/D. sacculus* complex were observed, suggesting that most of the cells identified were probably *D. sacculus*.

Based on morphometric characteristics, most of the cells identified here possess a more dorsally-convex hypothecal plate with dimensions (especially the ratio l/w) typical of *D. acuminata*, and even the length of the cells found here is considerably shorter than those described in the

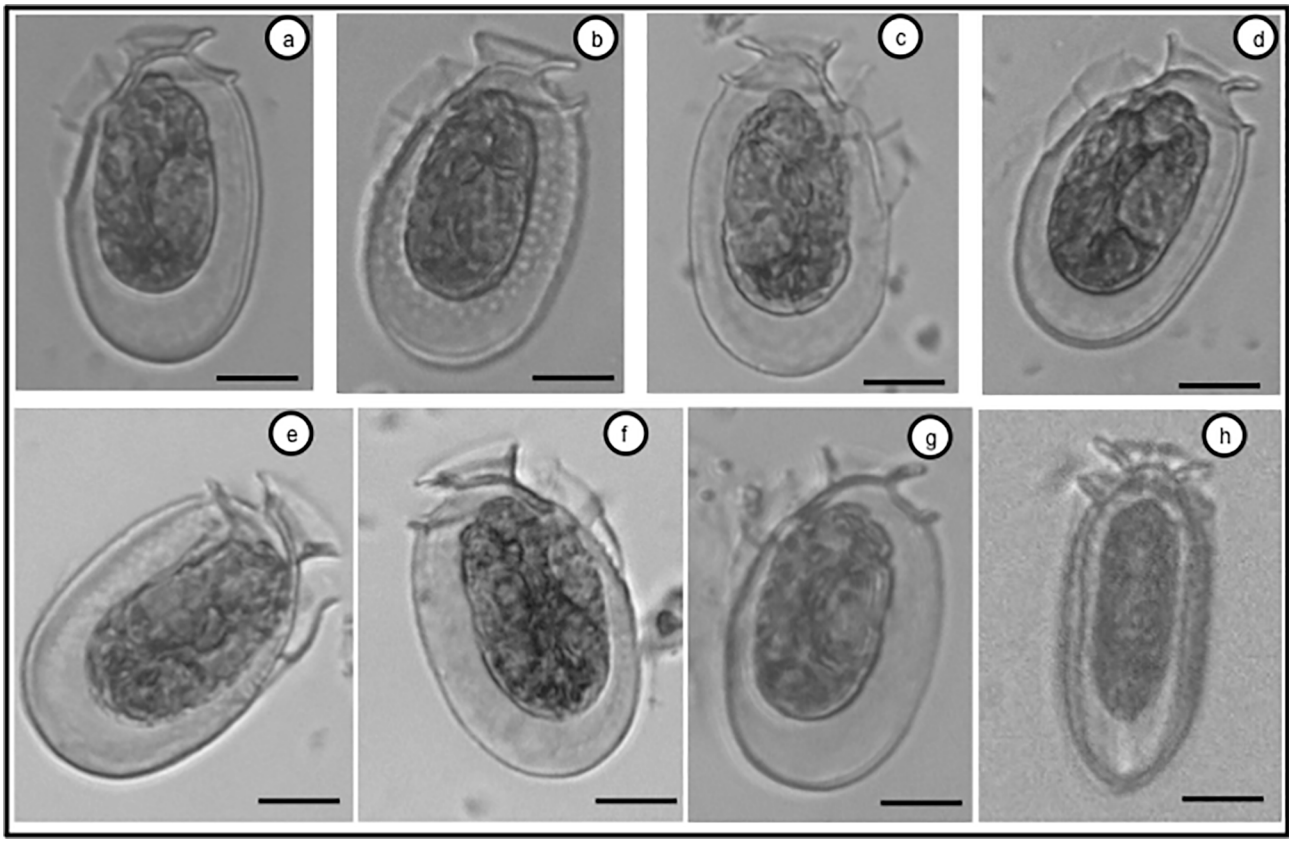

Figure 2. Lateral view (a-g) and hidden dimension (h) view of complete cells at Light Microscopy (LM) of *Dinophysis acuminata*/*D. sacculus* complex from Guanabara Bay samples. Scale bars: 10 µm.

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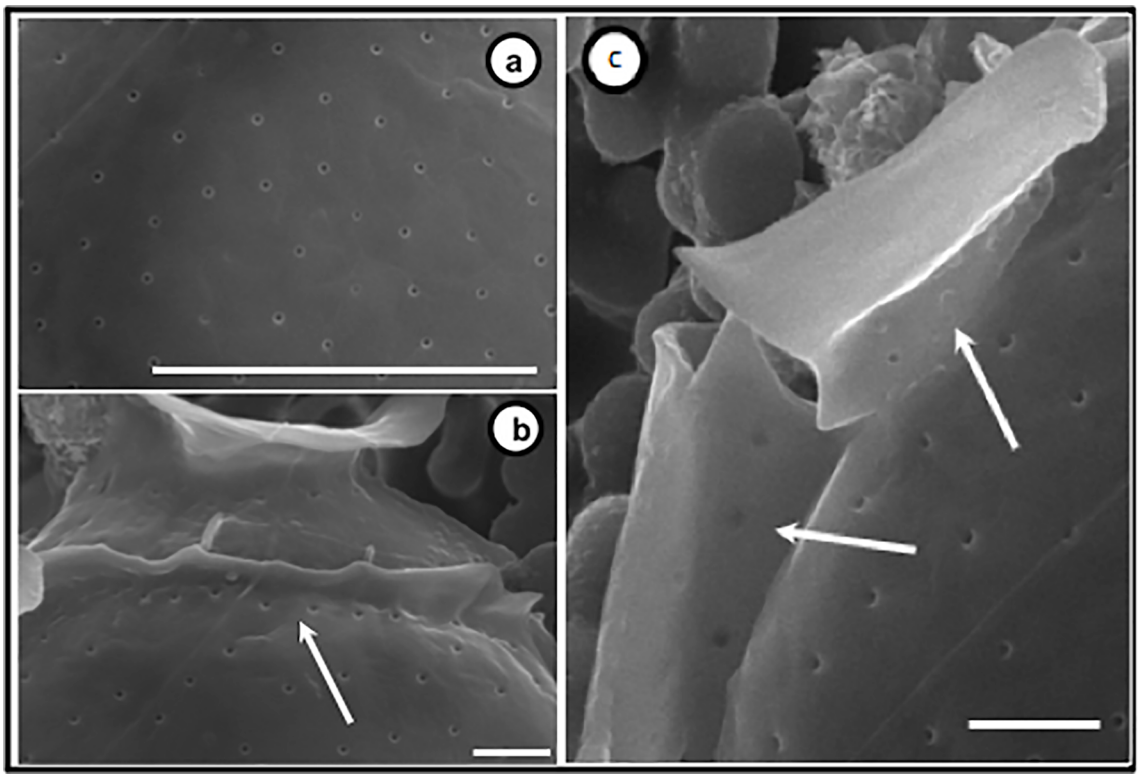

Figure 3. Details of the theca, scanning electron microscopy (SEM), of the cells of *Dinophysis acuminata*/*D. sacculus* complex from Guanabara Bay samples. Scale bars: a 10 µm; b-c 2 µm. Arrows indicate pores distribution.

literature (Zingone et al. 1998). On the other hand, the association with warmer and less saline estuarine waters was reported more often for *D. sacculus* than for *D. acuminata*, the latter being typical of colder waters. Nevertheless, both species of this complex were reported as potentially toxic even in low densities, as is the case for *D acuminata* (Zingone et al. 1998, Reguera et al. 2012, Reguera et al. 2014). In addition, nutrient loading can enhance both growth and toxicity of *Dinophysis* species (Hattenrath-Lehmann et al. 2015).

Thus, even without the ability to distinguish between the two species of the *D. acuminata/D. sacculus* complex, high densities of this complex would be monitored in highly eutrophic coastal areas, such as Guanabara Bay.

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Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

Author Contributions

José Juan Barrera-Alba: substantial contribution in the concept and design of the study, contributed also to data analyses and manuscript preparation.

Fernanda Reinhardt Piedras: substantial contribution in the concept and design of the study, contributed also to data analyses and manuscript preparation.

Gleyci Aparecida Oliveira Moser: substantial contribution in the concept and design of the study, contributed also to data analyses and manuscript preparation.

Carla Lucatelli Duarte: contributed to data analysis and interpretation.

Raquel Neves Tavares Lopes: contributed to data analysis and interpretation.

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